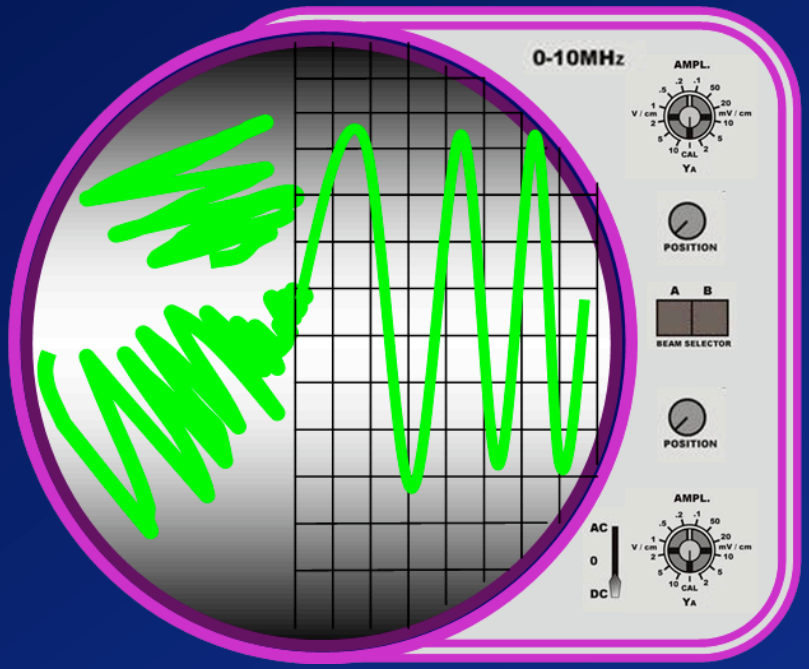
iGEM Jamboree
MIT – Nov 4th 2006



Engineering a Molecular Predation Oscillator

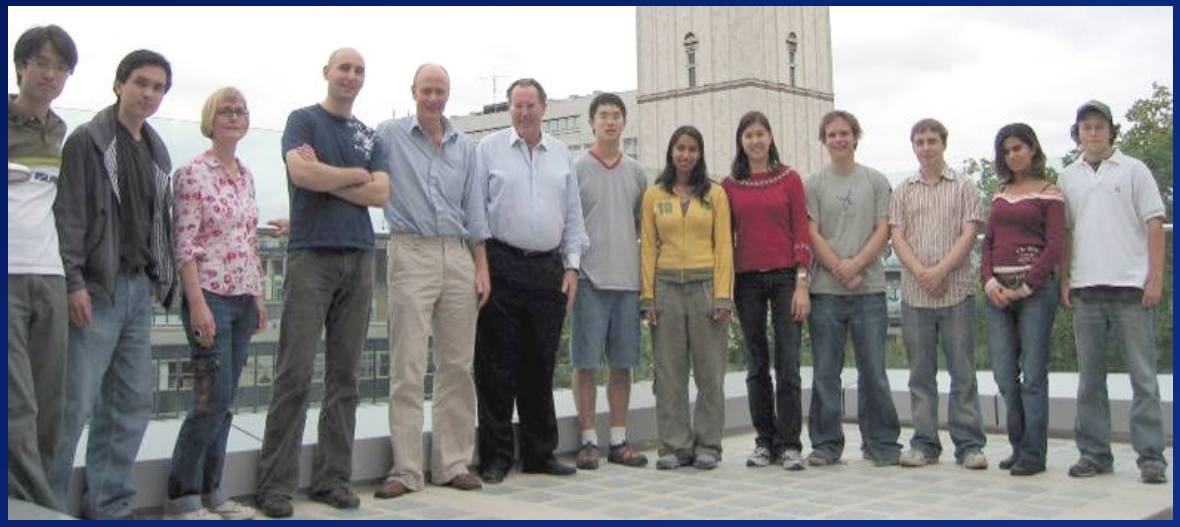
iGEM 2006 @ Imperial

Biomedical Engineers

Electrical Engineer

Biochemist

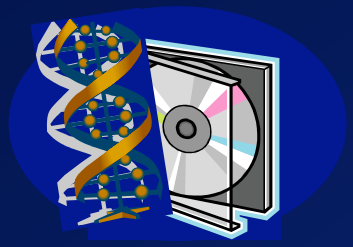
Biologists



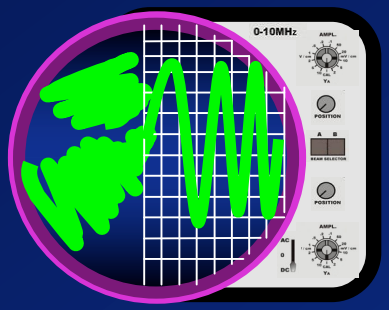
Biomedical Engineers

Biochemists

Project Ideas



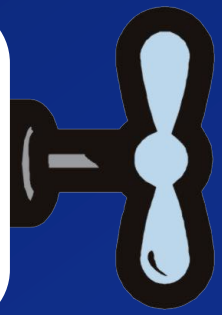
Bio-Memory



Oscillator

Bio-Clock

- Feasibility
- Originality of Design
- BioBrick Availability
- Future Impact

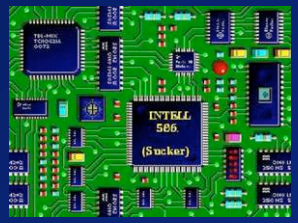


What is an Oscillator?

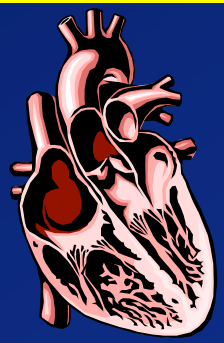
Our Definition

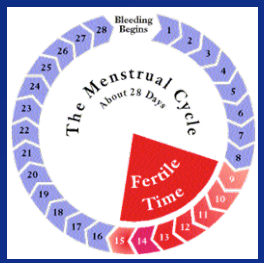
Device producing a periodic variation in time of a measurable quantity, e.g. amplitude.

Engineering



Biology

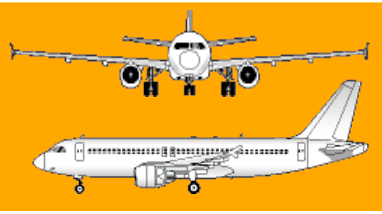




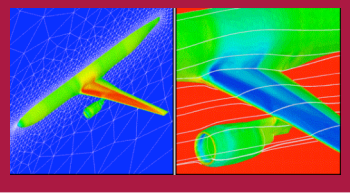
The Engineering Approach

Specifications

Design



Modelling



Implementation

Testing/Validation

Stand

Certified

ctice

The Main Challenges

Main challenges of past oscillators:

- Unstable
- Noisy
- Inflexible

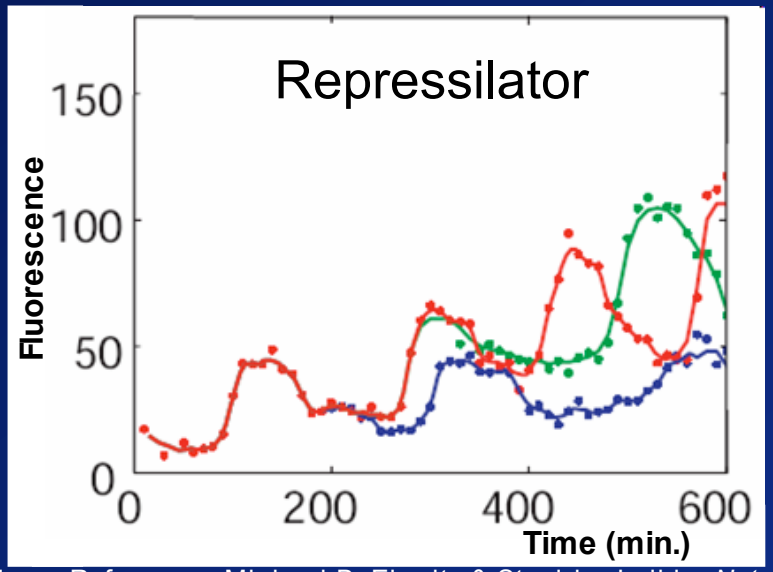


Figure Reference : Michael B. Elowitz & Stanislas Leibler *Nature* 2000

Requirement for a typical engineering oscillator

Our Specifications:

- Sustained Oscillations
- Stability: >10 periods
- High Signal to Noise Ratio SNR: High
- Flexibility: Controllable Oscillations Amplitude and Frequency
- Standardized Device for Easy Connectivity
- Modular Design
- Easy Connectivity

Our Initial Design Ideas

Based on

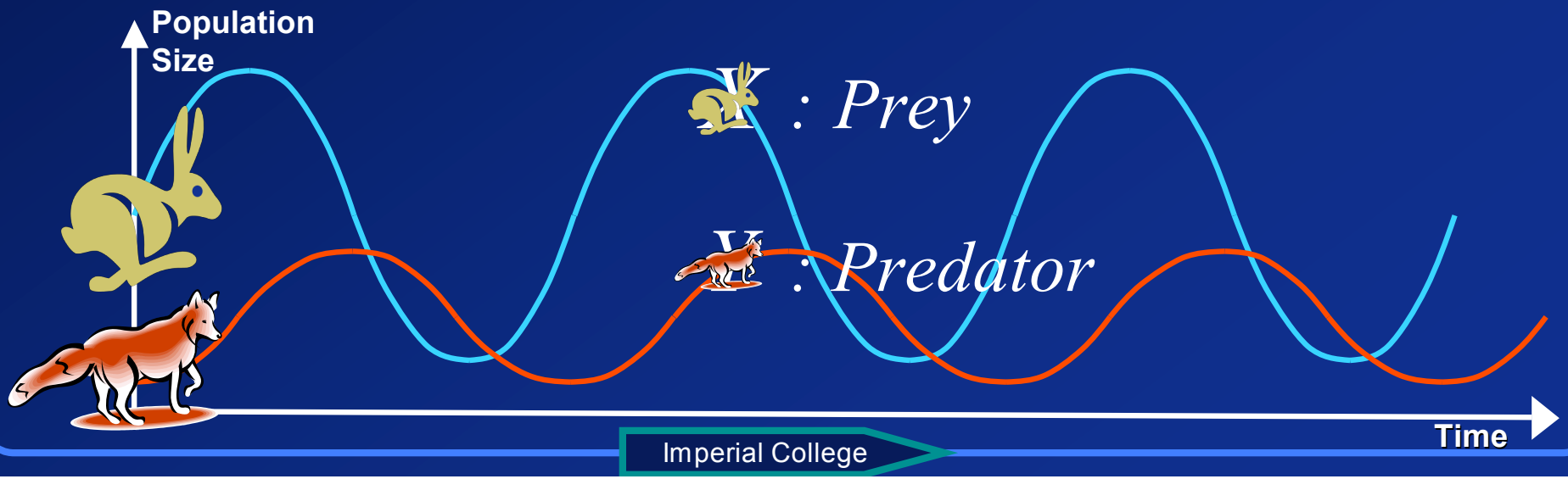
- Large populations of molecules to reduce influence of noise
- Oscillations due to population dynamics
- A well characterized model

Molecular Predator - Prey

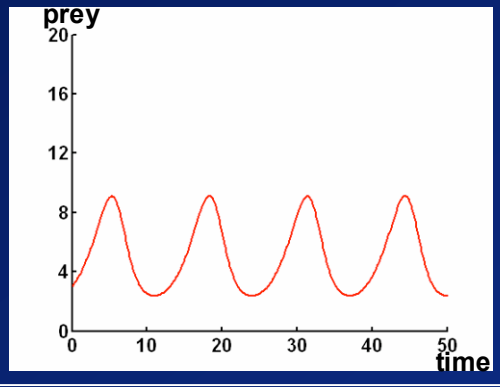
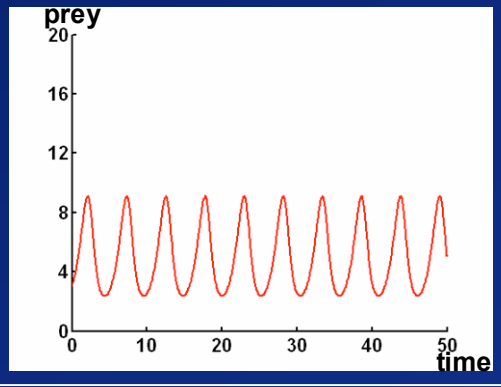
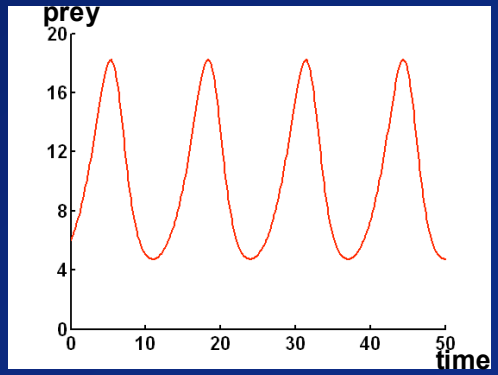
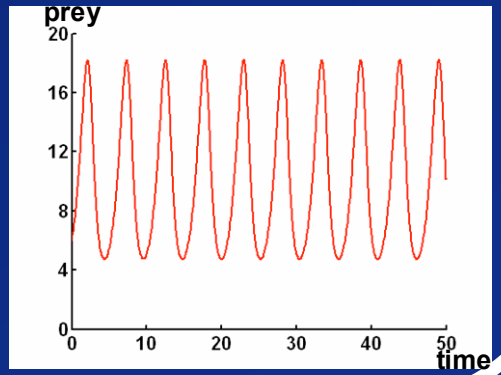
The Lotka-Volterra Model

$$\frac{dV}{dt} = \text{Prey Growth} - \text{Prey Killing by Predator}$$

$$\frac{dV}{dt} = \text{Predator Growth} - \text{Predator Death}$$



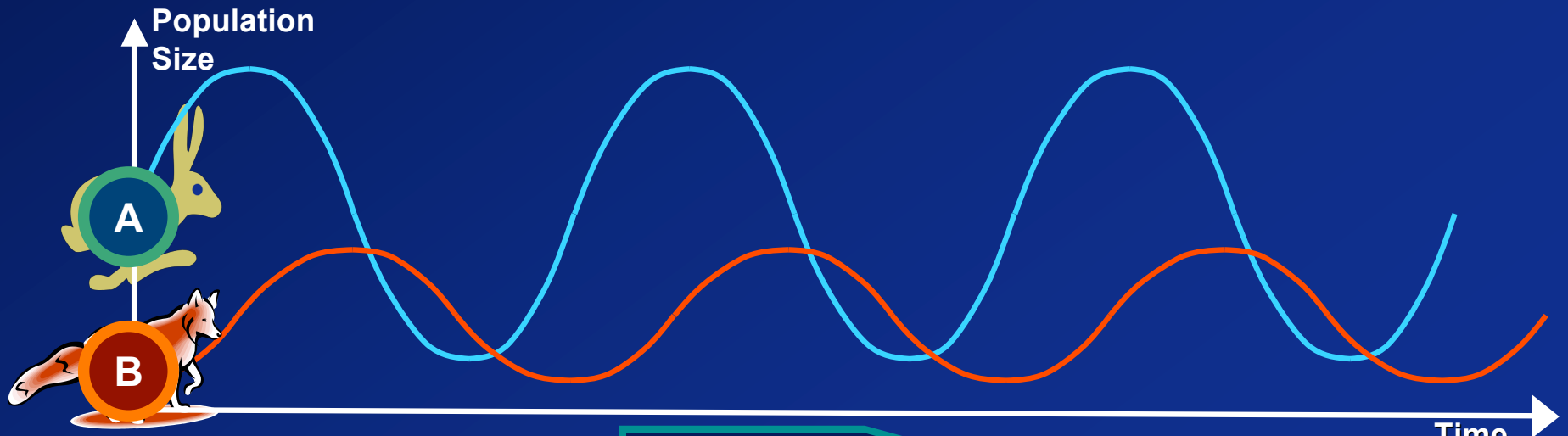
Typical LV Simulations

Graph of Prey vs. Time	Low Frequency	High Frequency
Small Amplitude		
Large Amplitude		

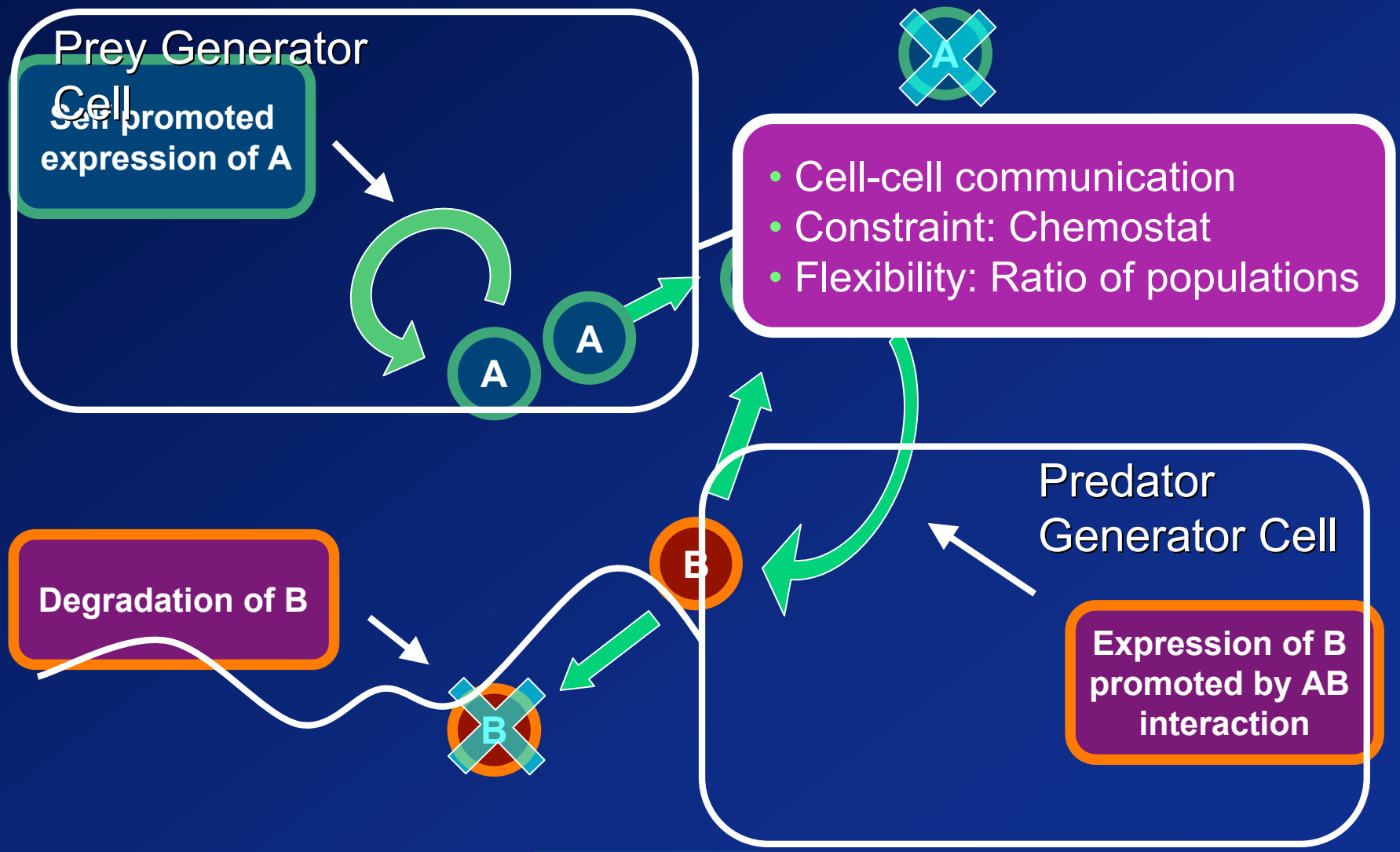
Required Biochemical Properties

$$\frac{d\,A}{dt} = \text{Self promoted expression of A} - \text{Degradation of A by B}$$

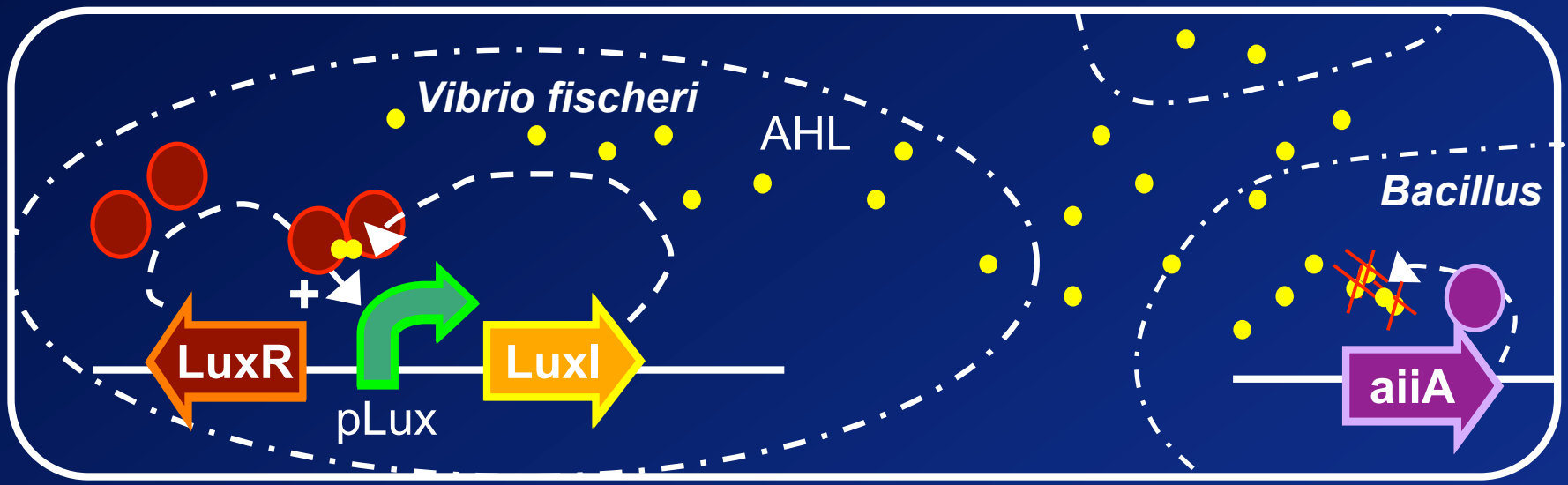
$$\frac{d\,B}{dt} = \text{Expression of B promoted by AB interaction} - \text{Degradation of B}$$



Molecular System



Quorum sensing/quenching



BioBricks available

BBa_C0062	Forms a complex with AHL to activate pLux	BBa_C0061	Makes AHL
pLux BBa_R0062	pLux Promoter	BBa_C0160	Degrades AHL
 BBa_F2620		AHL->Pops Receiver	

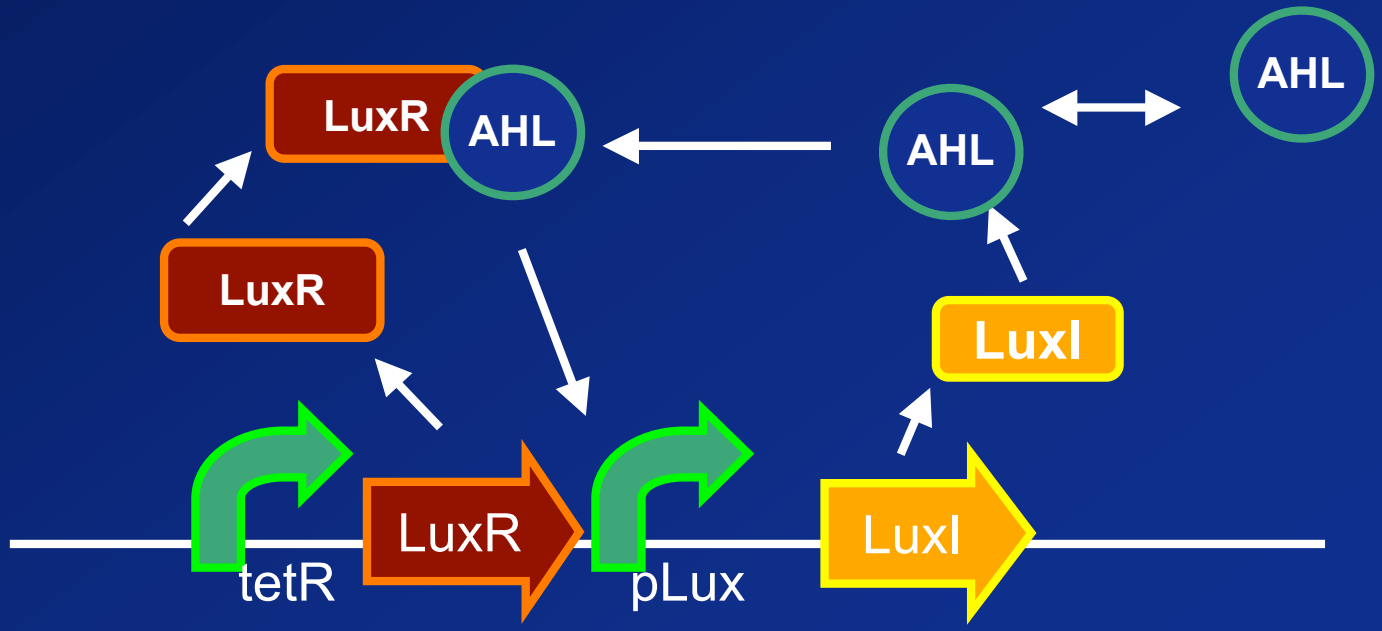
Designing the Prey Generator

Required Dynamic

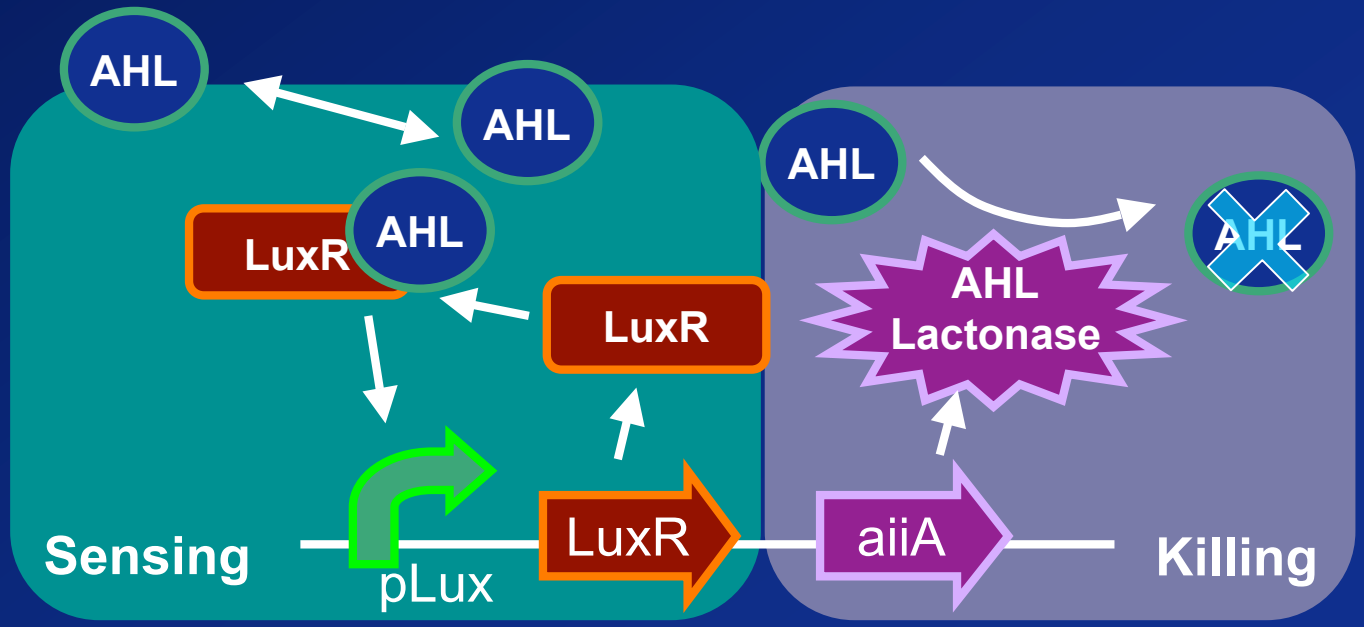
Self promoted expression of A ✓

Useful BioBricks

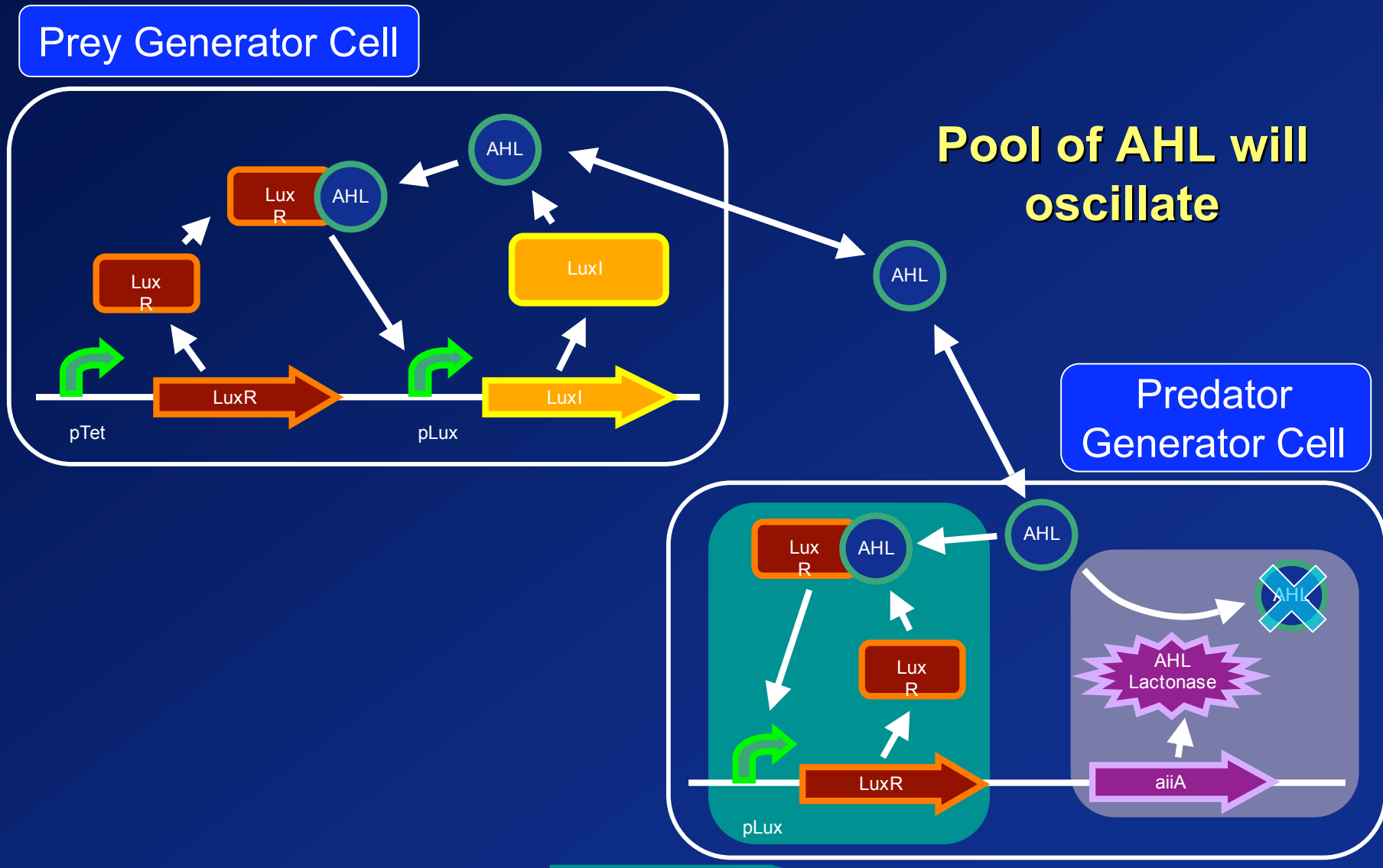
Final Construct



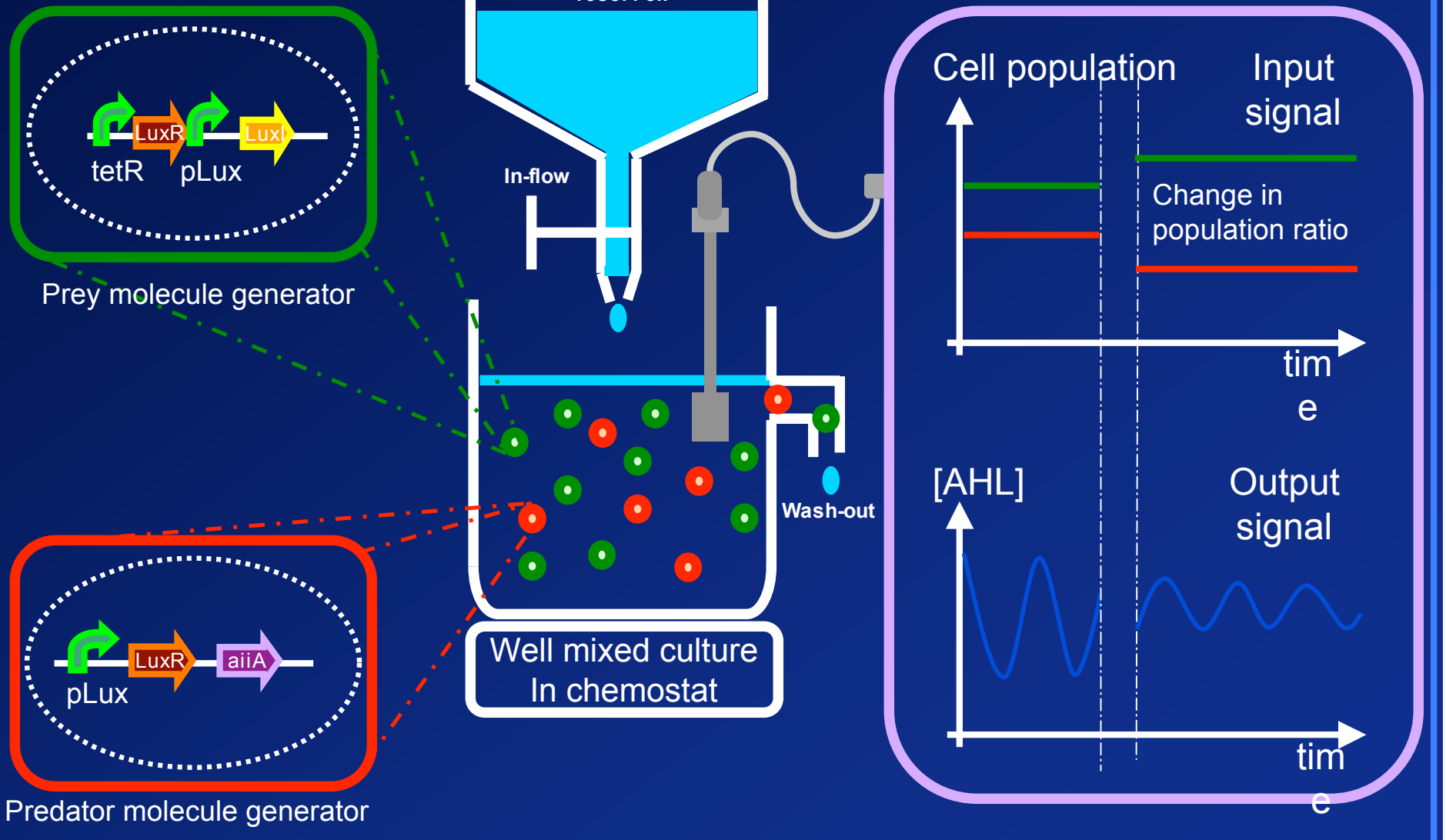
Designing the Predator Generator

Required Dynamic	Expression of B promoted by AB interaction ✓	Degradation of A by B ✓	Degradation of B ✓
Useful BioBricks	pLux R0062; LuxR C0062	aiiA C0060	Natural degradation
Final Construct			

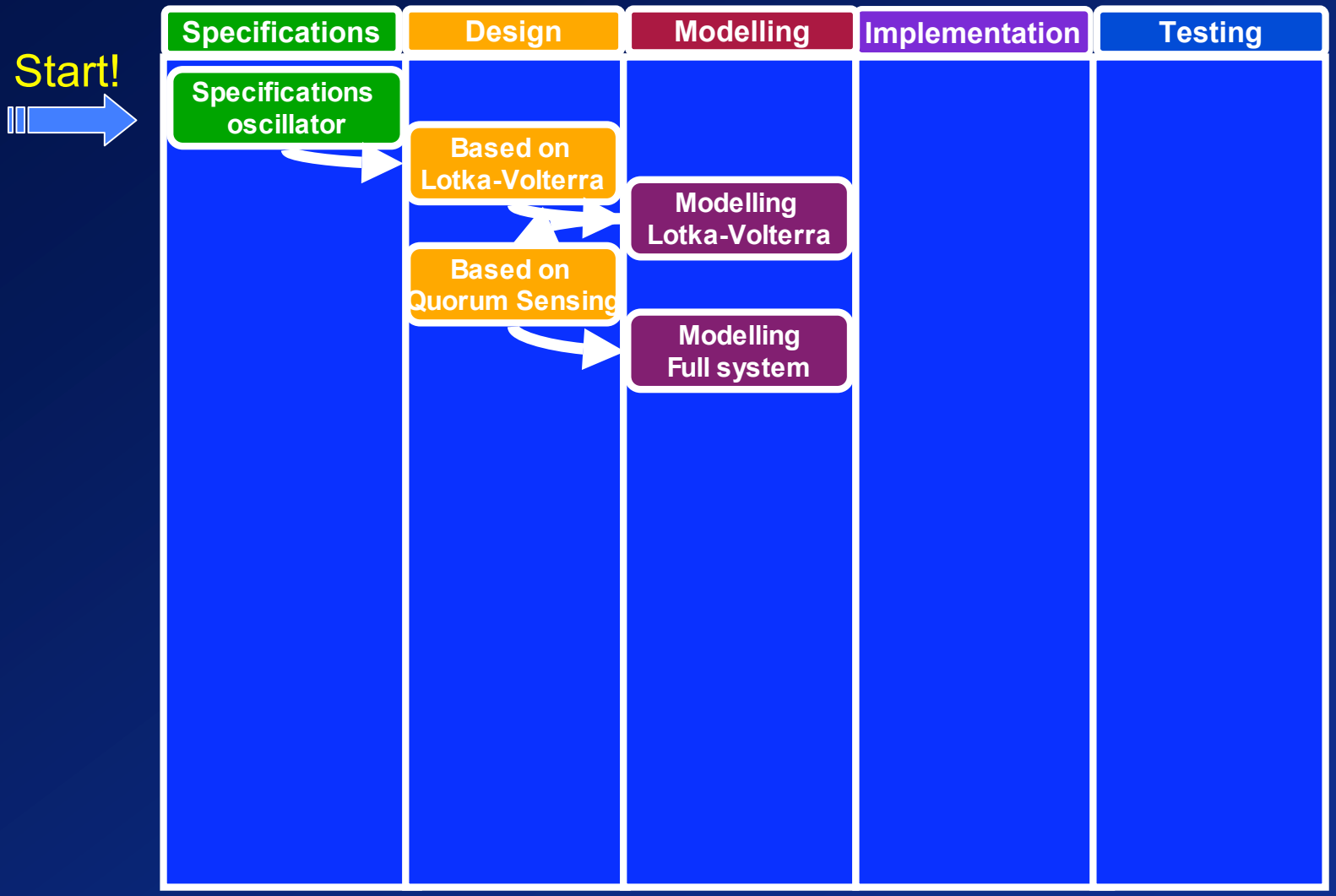
System Overview



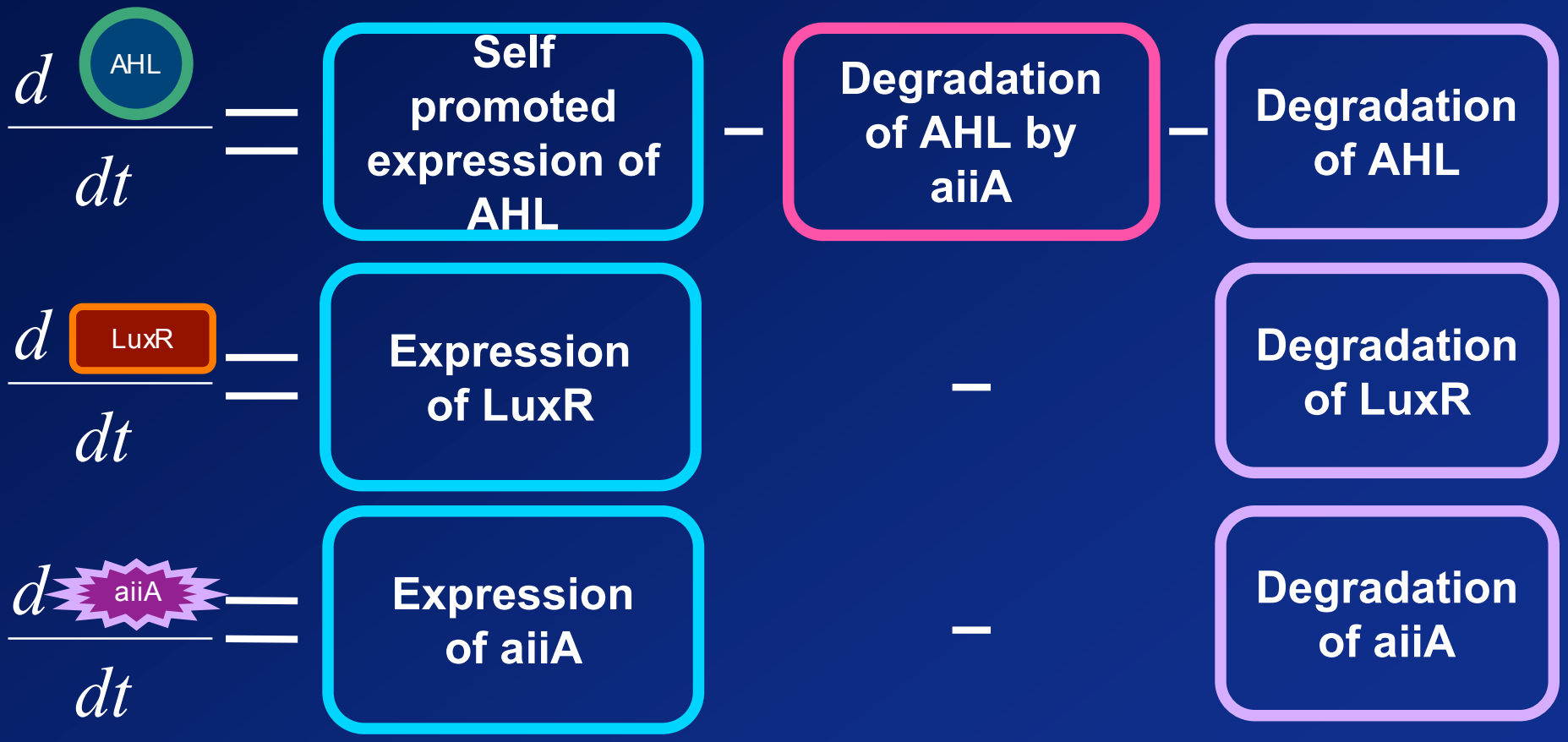
Full System set-up



Path to Our Goal



Modelling the Full System



Modelling the Full System

$\frac{d[AHL]}{dt}$ = Self promoted expression of AHL − Degradation of AHL by aiiA − Degradation of AHL

$\frac{d[LuxR]}{dt}$ = Expression of LuxR − Degradation of LuxR

$\frac{d[aiiA]}{dt}$ = Expression of aiiA − Degradation of aiiA

Modelling the Full System

Gene Expression

$$\frac{d[AHL]}{dt} = \frac{a[AHL]}{a_0 + [AHL]} - \text{Degradation of AHL by aiiA} - \text{Degradation of AHL}$$

$$\frac{d[LuxR]}{dt} = \frac{c[AHL][LuxR]}{c_0 + [AHL][LuxR]} - \text{Degradation of LuxR}$$

$$\frac{d[aiiA]}{dt} = \frac{c[AHL][LuxR]}{c_0 + [AHL][LuxR]} - \text{Degradation of aiiA}$$

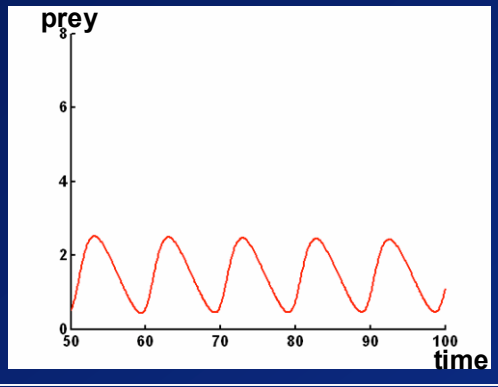
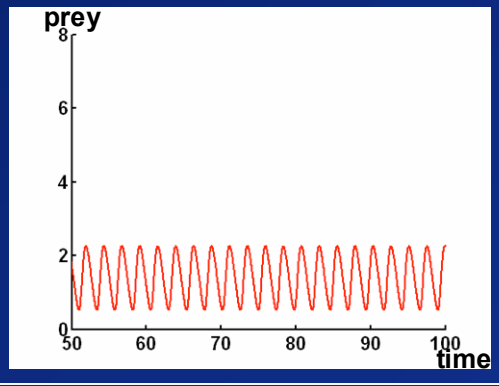
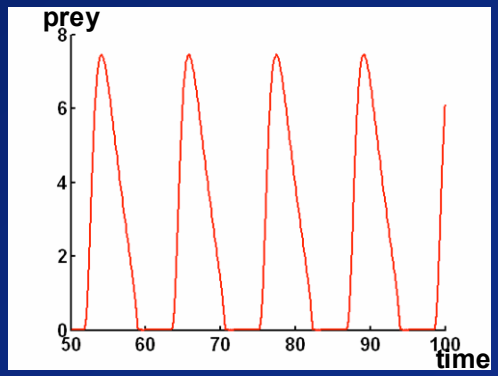
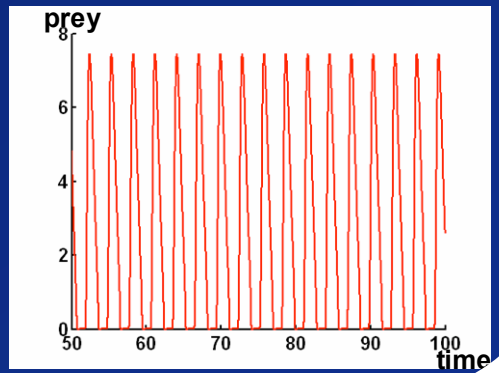
Modelling the Full System

	Gene Expression		Enzymatic Reaction	
$\frac{d[AHL]}{dt} =$	$\frac{a[AHL]}{a_0 + [AHL]}$	$-$	$\frac{b[aiiA][AHL]}{b_0 + [AHL]}$	$-$ Degradation of AHL
$\frac{d[LuxR]}{dt} =$	$\frac{c[AHL][LuxR]}{c_0 + [AHL][LuxR]}$		$-$	Degradation of LuxR
$\frac{d[aiiA]}{dt} =$	$\frac{c[AHL][LuxR]}{c_0 + [AHL][LuxR]}$		$-$	Degradation of aiiA

Modelling the Full System

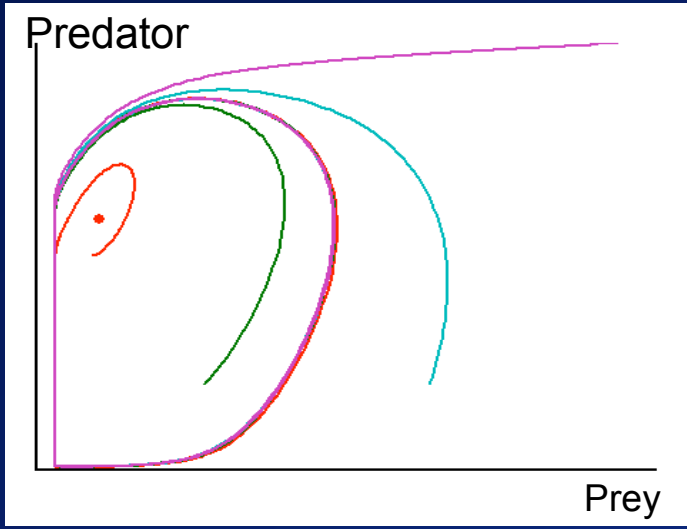
	Gene Expression	Enzymatic Reaction	Degradation
$\frac{d[AHL]}{dt} =$	$\frac{a[AHL]}{a_0 + [AHL]}$	$- \frac{b[aiiA][AHL]}{b_0 + [AHL]}$	$- e[AHL]$
$\frac{d[LuxR]}{dt} =$	$\frac{c[AHL][LuxR]}{c_0 + [AHL][LuxR]}$		$- d_1[LuxR]$
$\frac{d[aiiA]}{dt} =$	$\frac{c[AHL][LuxR]}{c_0 + [AHL][LuxR]}$		$- d_2[aiiA]$

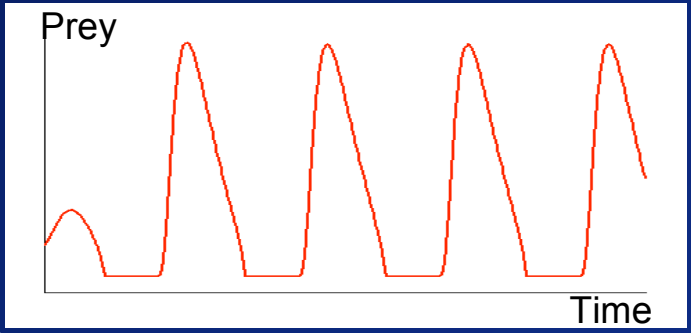
Full System Simulations

Graph of Prey vs. Time	Low Frequency	High Frequency
Small Amplitude		
Large Amplitude		

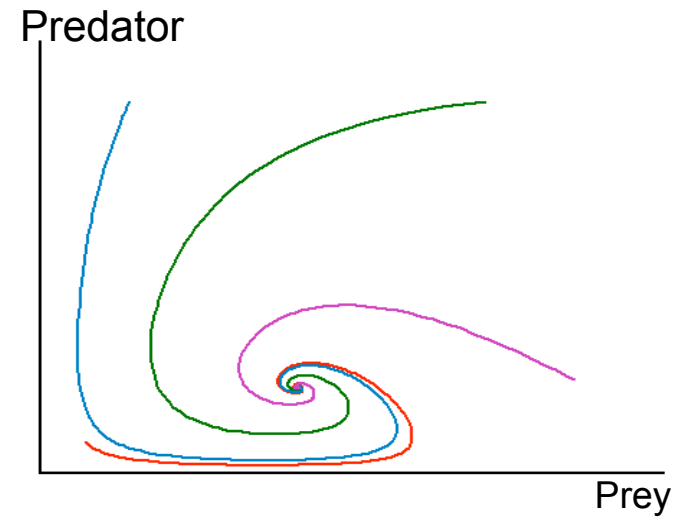
Typical System Behaviours

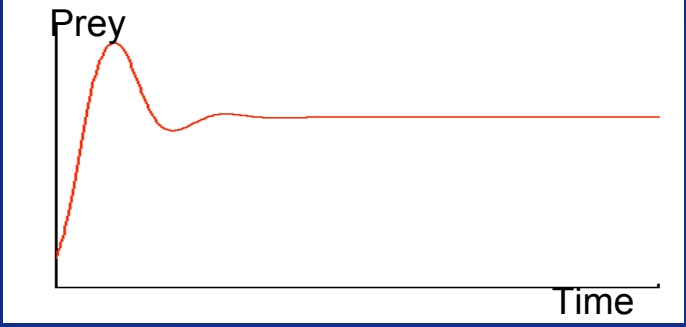
Oscillations with limit cycles

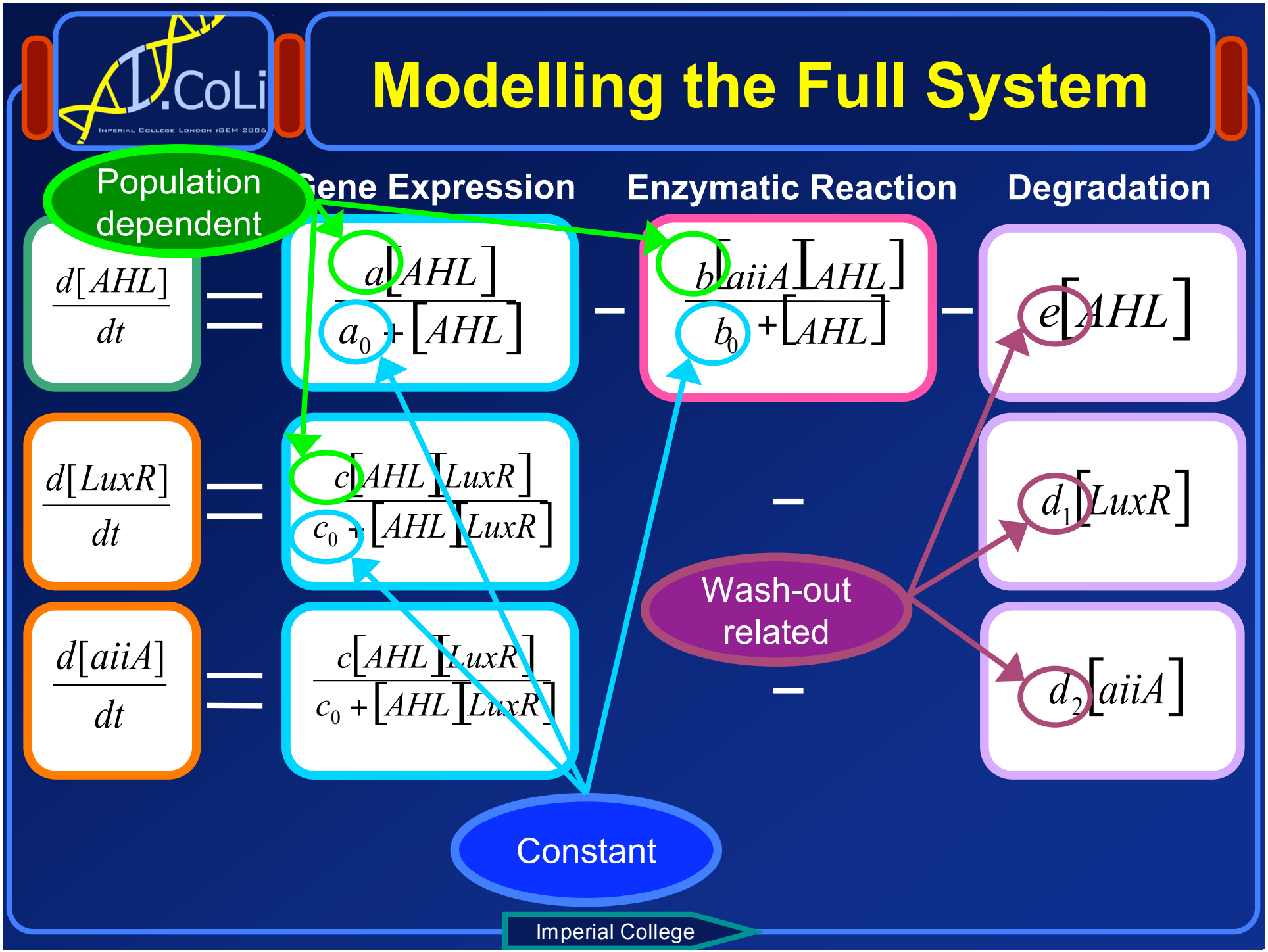




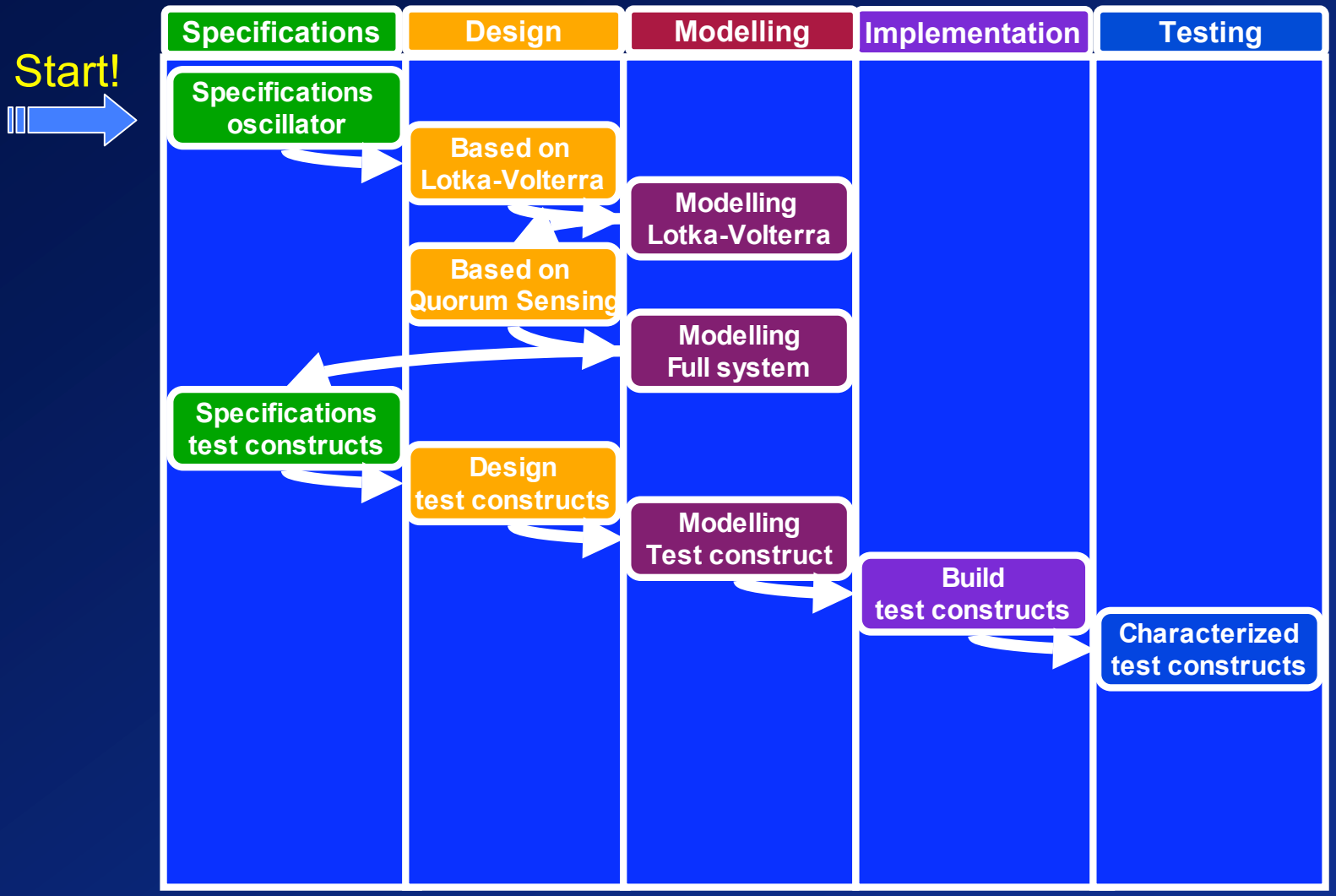
No oscillations





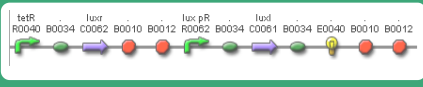

Modelling the Full System
Population dependent
Gene Expression
Enzymatic Reaction
Degradation
$\frac{d[AHL]}{dt} = \frac{a[AHL]}{a_0 + [AHL]} - \frac{b[aiiA][AHL]}{b_0 + [AHL]} - e[AHL]$
$\frac{d[LuxR]}{dt} = \frac{c[AHL][LuxR]}{c_0 + [AHL][LuxR]} - d_1[LuxR]$
$\frac{d[aiiA]}{dt} = \frac{c[AHL][LuxR]}{c_0 + [AHL][LuxR]} - d_2[aiiA]$
Wash-out related
Constant
Imperial College

Path to Our Goal

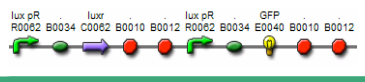


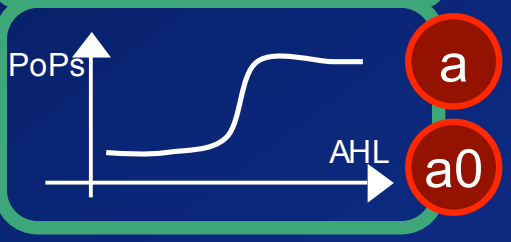
Breaking Down the Complexity

Prey Generator

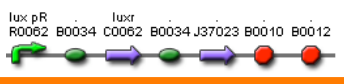


Prey Sensing

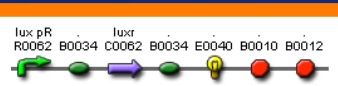


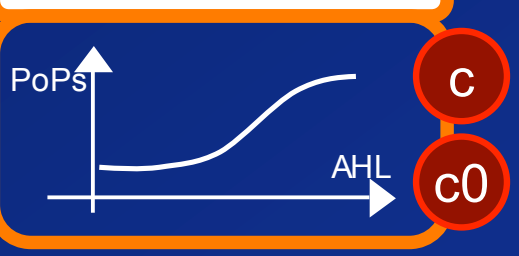


Predator Generator

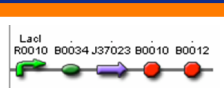


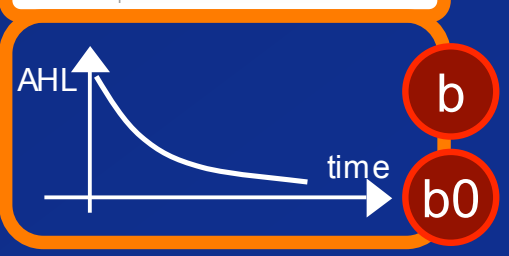
Predator Sensing





Predator Killing





Characterization Predator Sensing

Test part

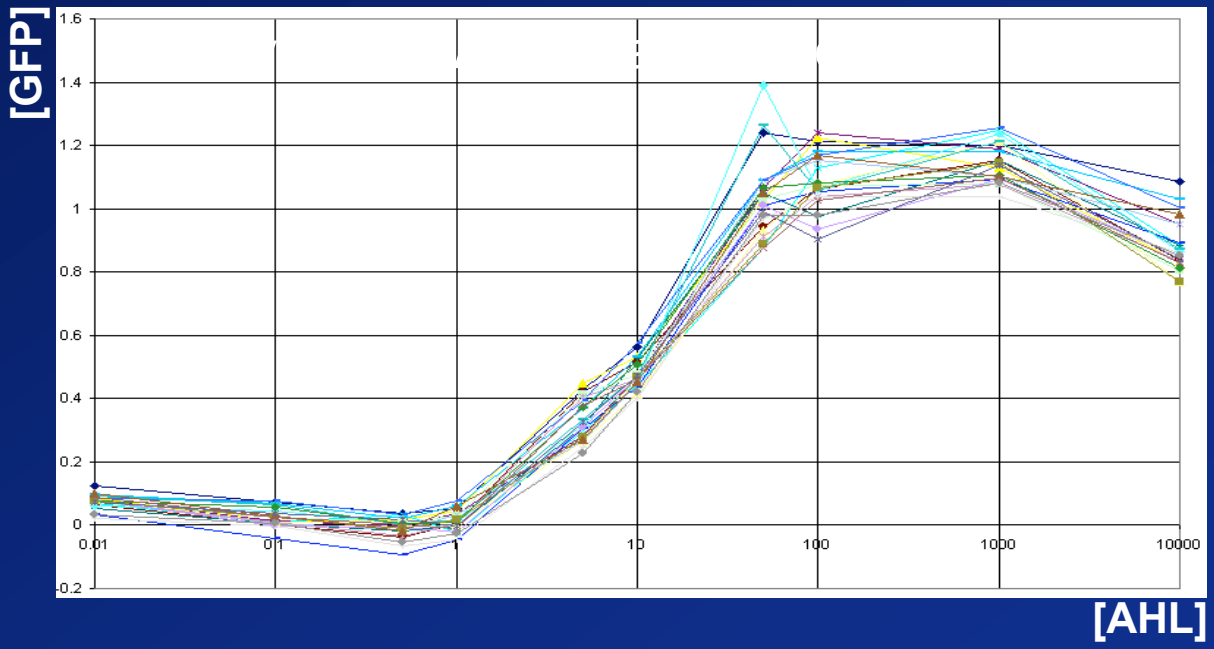
↓

Predictive model transfer function

↓

Experimental data

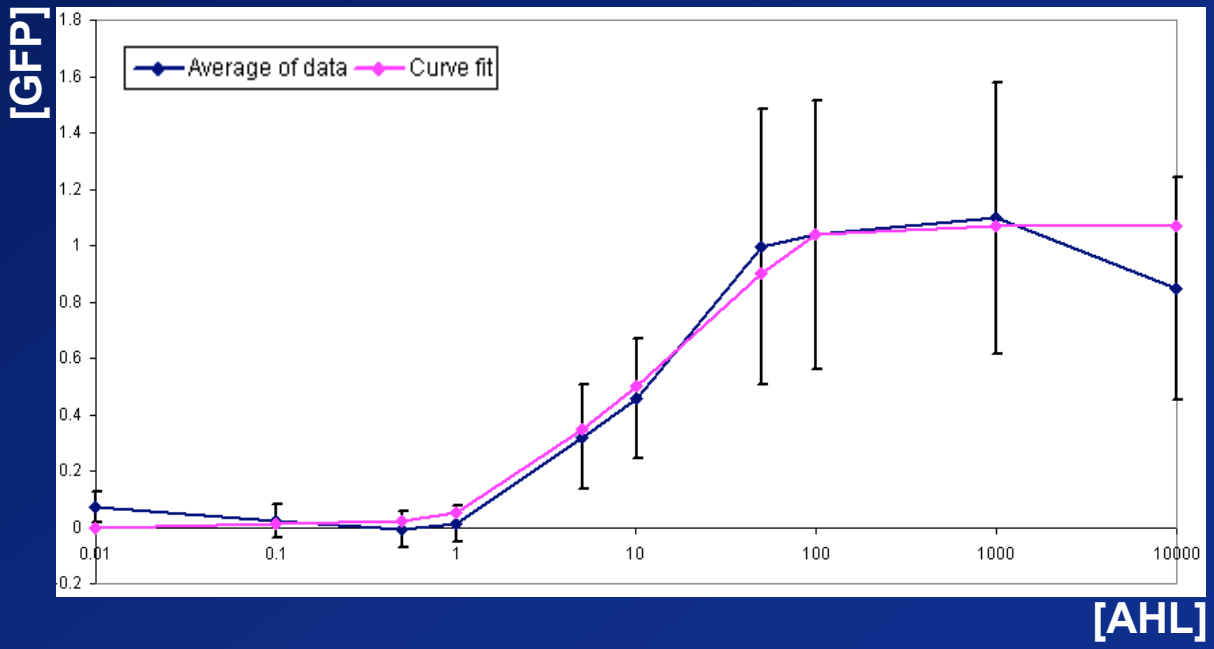
Experimental Data



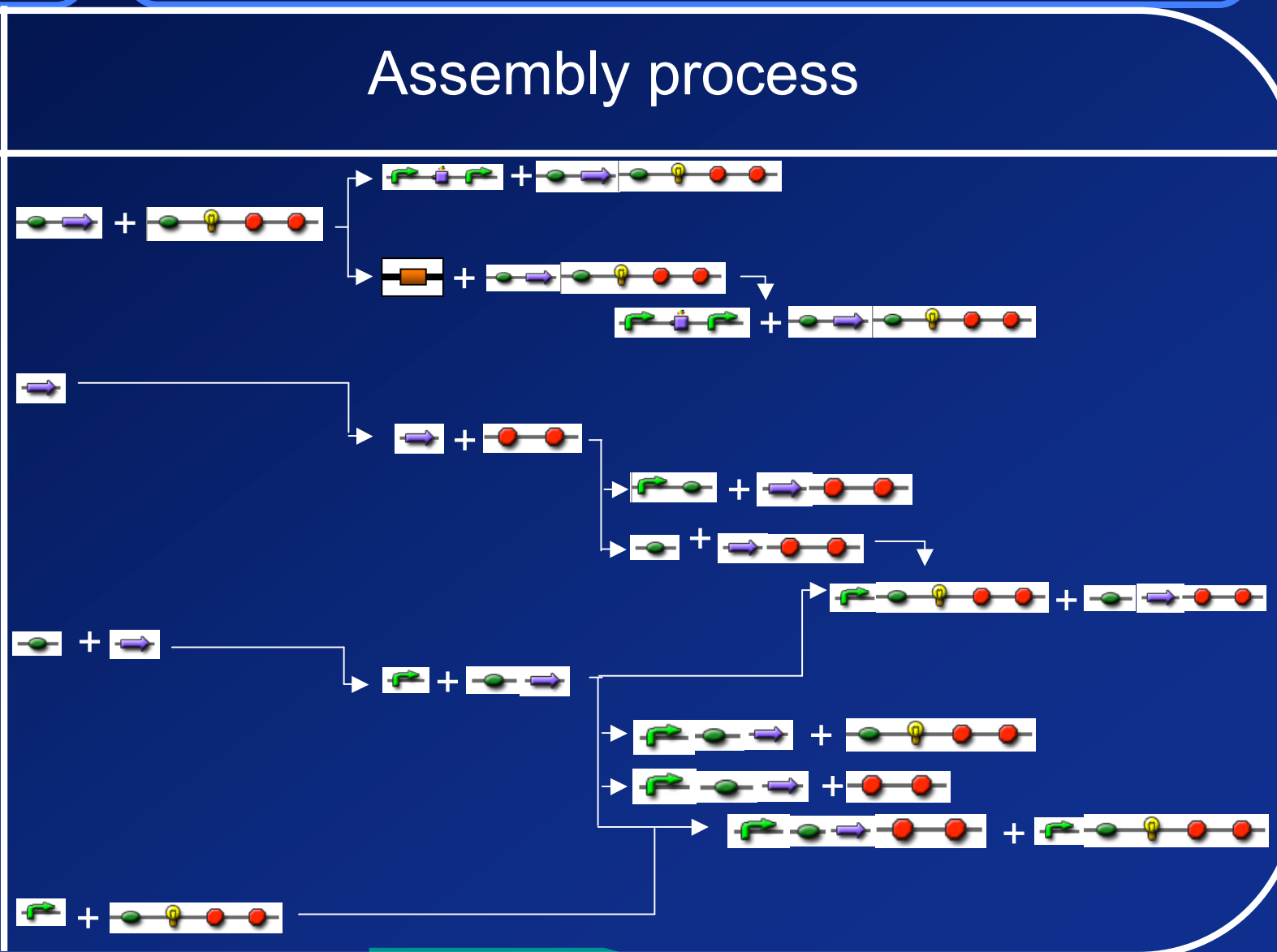
Characterization Predator Sensing

- Test part
- Predictive model transfer function
- Experimental data
- Fitting model to data
- Parameter extractions

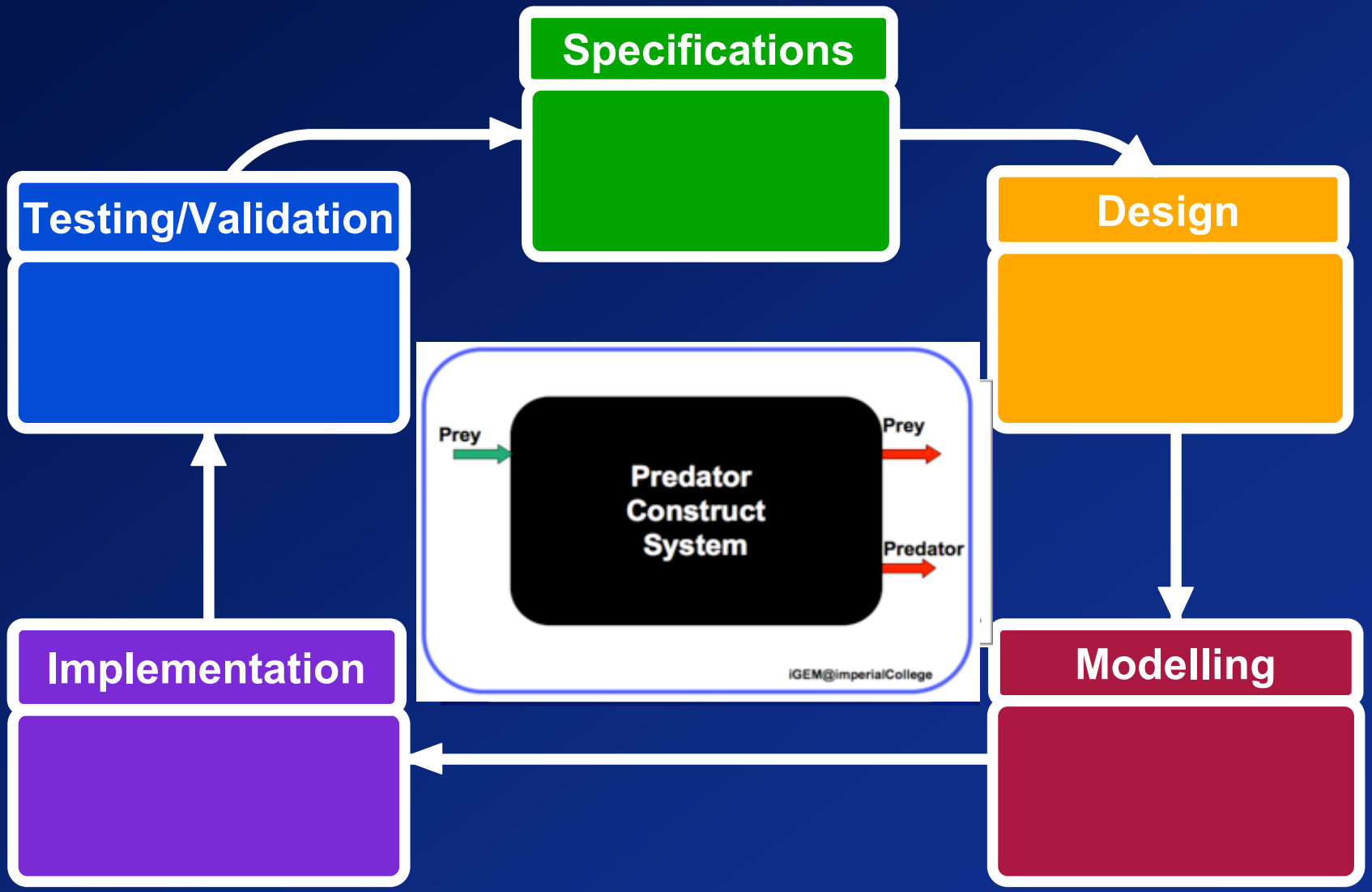
Average with variance and curve fitting



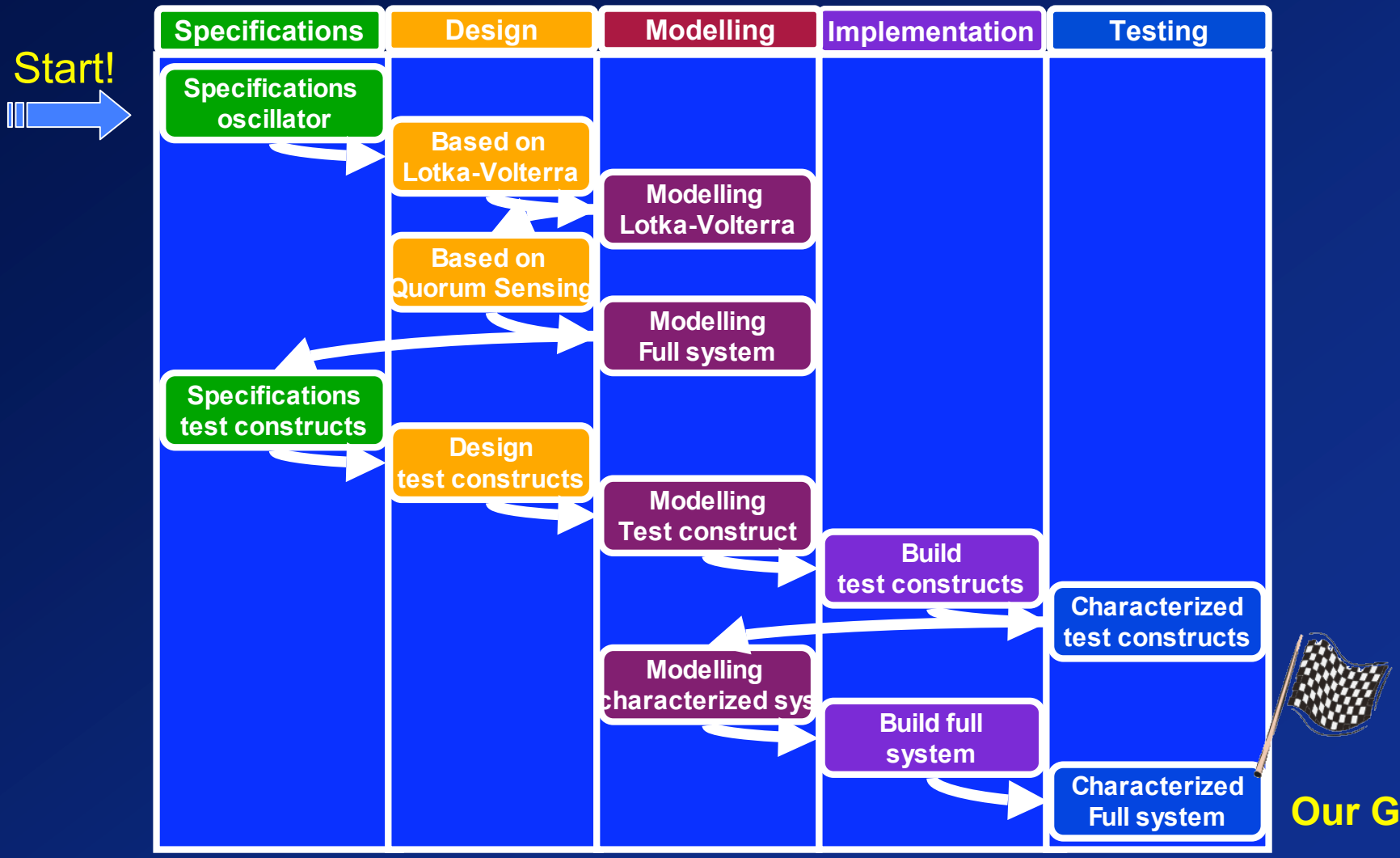
Implementation



On Our Experience



Path to Our Goal



Contributions to the Registry

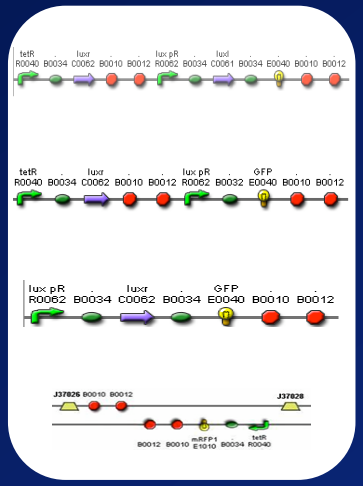
Functional Parts

Final Prey
J37015

Sensing Prey
T9002

Sensing Predator
J37016

Cre/Lox
J37027

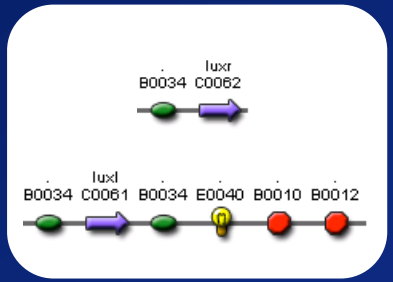


Built	Sequenced	Tested	Characterized	Documented
✓	✓	✓	✓	✓
		✓		✓
✓	✓	✓	✓	✓
✓	✓	✓		✓

Intermediate Parts

J37033

J37034



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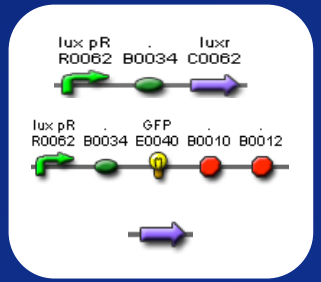
✓	✓

J37019

J37032

J37023



Built	Sequenced
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✓	✓
✓	✓

Our Wiki

Imperial College London

THE I.CoLi REPORTER

The newsletter of Imperial College London iGEM 2006

Our Contributions | To Do List | Biological Oscillator Parts | Modelling | Protocols | Resources

Breaking News [edit]

- 30 Oct 2006: 300+ pages documentation on OWW. New iGEM page
- 27 Oct 2006: Team Poloshirts arrived.
- 26 Oct 2006: BioBricks sent to M.I.T.
- 25 Oct 2006: Presentation of the iGEM 2006 project at the Bioengineering Departmental Seminar.
- News Archive

Location of our visitors

Visits since 6 Aug 2006

Updated daily

Imperial College London iGEM Project(s) 2006 edit

Engineering a Molecular Predation Oscillator.

PoPs Blocker | Biological to Electrical Interface

Celebrities [edit]

Get to know the I.Coli team and their advisors:

The Undergrads [show]

The Advisors [show]

Acknowledgements

Activities [edit]

- Event Calendar
- MIT Jamboree
- BioSysBio Conference, UK
- UK iGEM Teams Meeting in Cambridge

In the Wetlab [edit]

- Lab Notebook
- Protocols
- Lab Status
- Testing
- Sequencing of Parts
- Primers

Education [edit]

- Brainstorming
- Journal Club
- Lecture Notes
- Bibliography/Papers
- Resources

Entertainment [edit]

- Photos
- Logo & Team Shirts
- Inspirational Quotes
- Project Discussion
- Sandbox

- Documentation
- Communication
- Organization

http://openwetware.org/wiki/IGEM:IMPERIAL/2006

Thank You

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- David Featherbe
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- **Faculty of Natural Sciences**